Kinetic and Thermodynamic Analysis of Two Carboxymethylcellulases from *Macrotermes subhyalinus* Little Soldier

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Abstract

Optimization of thermal processes relies on adequate degradation kinetic models to warrant food safety and quality. The knowledge on thermal inactivation of enzymes is necessary to allow their proper utilization in food industry and technology applications, enabling the reduction of heating times and optimization of heating temperatures. In this work, the kinetic of thermal inactivation was studied for the previously characterized carboxylmethylcellulases Ab-CX1 and Ab-CX2 from *Macrotermes subhyalinus* little soldier. Samples of carboxymethylcellulases were treated at different time-temperature combinations in the range of 5-60 min at 50-65°C and the kinetic and thermodynamic parameters for carboxymethylcellulases were calculated. Results showed that inactivation followed a first-order reaction with k-values between 0.0103 ± 0.0003 to 0.1217 ± 0.0005 and 0.0149 ± 0.0007 to 0.0416 ± 0.0003 min^{-1} for Ab-CX1 and Ab-CX2, respectively. At high temperatures, Ab-CX2 was less resistant, with a significant decrease in residual activity compared to Ab-CX1. The *D*- and *k*-values decreased and increased, respectively, with increasing temperature, indicating faster inactivation of carboxymethylcellulases. Activation energy (*Ea*) and *Z*-values were estimated to 76.74 ± 1.98 kJ.mol^{-1} and 24.21 ± 1.92 °C for Ab-CX1, 62.80 ± 2.05 kJ.mol^{-1} and 33.33 ± 2.78 °C for Ab-CX2. Thermodynamic parameters ($\Delta H^{\#}$, $\Delta S^{\#}$ and $\Delta G^{\#}$) were also calculated. The high value obtained for the variation in enthalpy of activation indicates that a high amount of energy is required to initiate denaturation, probably due to the molecular conformation of carboxymethylcellulases. All results suggest that both carboxymethylcellulases are relatively resistant to long heat treatments up to 50°C.

Keywords: carboxymethylcellulases, kinetic parameters, *Macrotermes subhyalinus*, thermal stability, thermodynamic parameters

1. Introduction

Cellulose is the major component of plant biomass. It is a linear biopolymer consisting of anhydroglucopyranose molecules (glucose) connected by β-1,4-glycosidic bonds (Hong et al., 2012) and its molecules (polymer) vary widely in length and are usually arranged in bundles or fibrils (Haruta et al., 2002; Walsh, 2002). In nature, the hydrolysis of cellulose requires the synergistic action of cellulases, which include: endoglucanase, exoglucanase and β-glucosidase (Fagbohunka et al., 2017). In recent decades, the interest in hemicellulases and cellulases has increased due to the ethanol production from lignocellulosic residues (De Almeida et al., 2011). Thus, cellulases have several potential applications in industries like biofuel, textile, paper, feeds, fruits and vegetables (Zaldivar et al., 2001; Fagbohunka, et al., 2017). These enzymes are also used for non-specific cleavage of chitosan to release oligosaccharides with low molecular mass (Xia et al., 2008). Therefore, cellulases have been widely used in food and pharmaceutical industries and also for controlling environmental pollution (Jabbar et al., 2008; Niaz et al., 2011). In addition, cellulases have a wide range of industrial applications such as the treatment of starch processing, animal feed production, grain alcohol fermentation, fermentation, malting and brewing, extraction of fruit and vegetable juices, pulp and paper industry and textile industry (Ögel et al., 2001; Adsul et al., 2007; Kaur et al., 2007; Papinutti & Forchiassin, 2007). Based on the importance of lignocellulose-degrading enzymes, many

authors have attempted to purify and characterize these enzymes in some microorganisms (Lama et al., 2004; Ninawe et al., 2008; Gaffney et al., 2009) and insects (Séa et al., 2006; Binate et al., 2008; Arakawa et al., 2009; Blei et al., 2010; Fagbohoun et al., 2012; Fagbohunka, et al., 2017).

But, for industrial applications, enzymes must be stable under process conditions. Generally, enzymes are preferred over chemical catalysts. Therefore, thermophilic microorganisms are believed to be potentially good alternative sources of thermostable enzymes (Egas et al., 1998). Therefore, thermostable enzymes have been reported to have higher stability to organic solvent, alkaline and acidic pH and detergents (C. Vieille et al., 1996). Other benefits include enhancement of reaction rate constant, increasing the diffusion rate as the medium viscosity decreases with an increasing temperature (Kumar & Swati, 2001). Thus, one of the ways to identify enzymes which are thermally stable is to exploit natural sources such as both thermophilic and mesophilic organisms. Thermophilic organisms are known to produce enzymes having higher thermostability than those derived from their mesophilic counterparts (George et al., 2001). In this regard, comparative studies of thermophilic and mesophilic enzymes have demonstrated that weak interactions such as hydrogen bonds (Macedo-Ribeiro et al., 1996), disulfide bonds (Hopfner et al., 1999), ion pairs (Vetriani et al., 1998), salt bridges (Criswell et al., 2003), hydrophobic interactions (Elcock, 1998) and compactness (Russell et al., 1997) are important for stability. Therefore, enhancement of the structural stability of enzymes is of great importance for their application in several industrial processes. Thus, any process that enhances the structural stability and reaction rate of enzymes has a favorable impact on their industrial application (Sousa, 1995; Matsumoto et al., 1997).

In previous study, we purified to homogeneity monomeric carboxymethylcellulases from little soldier of termites *Macrotermes subhyalinus* (Fagbohoun, et al., 2012). However, there is no report concerning thermal stability of these cellulases. Thus, the knowledge on kinetics of thermal inactivation of two carboxymethylcellulases Ab-CX1 and Ab-CX2 is important to allow their suitable utilization as biocatalysts in industry. Therefore, the aim of this work was to evaluate the stability, then to determinate kinetic and thermodynamic parameters of carboxymethylcellulases Ab-CX1 and Ab-CX2.

2. Material and Methods

2.1 Enzymes

Carboxymethylcellulases (Ab-CX1 and Ab-CX2) used in this study were previously purified from little soldier of *Macrotermes subhyalinus* (Fagbohoun, et al., 2012; Fagbohoun, 2013). Theses enzymes were homogeneous on polyacrylamide-gel electrophoresis in the absence of sodium dodecyl sulphate (SDS).

2.2 Carboxymethylcellulases Assays

Under the standard test conditions, cellulase activity was assayed spectrophotometrically by measuring the release of reducing sugars from carboxymethylcellulose (CMC) (Fagbohoun, et al., 2012; Fagbohoun, 2013). The essay mixture (0.38 mL) containing 0.2 mL of CMC (0.5%, w/v) in 20 mM sodium acetate buffer (pH 5.0) with 0.1 mL enzyme solution, was incubated at 45°C for 30 min. The reaction was stopped by adding 0.3 mL of dinitrosalicylic acid solution and heating for 5 min in boiling water bath. The absorbance was measured at 540 nm after cooling on ice for 5 min.

One unit (U) of enzyme activity was defined as the amount of enzyme capable of releasing one μmol of reducing sugar per min under the defined reaction conditions. Specific activity was expressed as units per mg of protein (U/mg of protein).

2.3 Protein Determination

Protein was determined according to Lowry method (Lowry et al., 1951) using bovine serum albumin as standard.

2.4 Thermal Inactivation

Thermal inactivation of each carboxymethylcellulase was investigated at various constant temperatures from 50 to 65°C after exposure to each temperature for a period of 5 to 60 min. The enzyme was heated in sealed tubes, which was incubated in 100 mM sodium acetate buffer (pH 5.0) in a thermostatically controlled water bath. Tubes were withdrawn at each time intervals and immediately immersed in an ice bath, in order to stop heat inactivation. The residual enzymatic activity, determined in both cases at 37°C under the standard test conditions, was expressed as percentage activity of zero-time control of the untreated enzyme.

2.5 Kinetic Data Analysis

First-order kinetic has been reported to describe thermal inactivation of carboxymethylcellulases (Guiavarc'h et al., 2002). The integral effect of inactivation process at constant temperature, where the inactivation rate constant is independent of time, is given in Eq. 1:

$$ln\ (At/A0) = -\ kt \tag{1}$$

where, At is the residual enzyme activity at time t (min), Ao is the initial enzyme activity, k (min^{-1}) is the inactivation rate constant at a given condition. k-values were obtained from the regression line of $ln\ (At/Ao)$ versus time as slope.

D-value is defined as the time needed, at a constant temperature, to reduce the initial enzyme activity (Ao) by 90 %. For first-order reactions, the D-value is directly related to the rate constant k (Eq. 2) (Stumbo, 1973; Espachs-Barroso et al., 2006):

$$D = 2.303/k \tag{2}$$

The Z-value (°C) is the temperature increase needed to induce a 10-fold reduction in D-value (Stumbo, 1973). This Z-value follows the Eq. 3:

$$log\ (D1/D2) = (T2 - T1)/Z \tag{3}$$

where, $T1$ and $T2$ are the lower and higher temperatures in °C or K. Then, $D1$ and $D2$ are D-values at the lower and higher temperatures in min, respectively.

The Z-values were determined from the linear regression of log (D) and temperature (T).

2.6 Thermodynamic Analysis

The Arrhenius equation is usually utilized to describe the temperature effect on the inactivation rate constants and the dependence is given by (Eq. 4 or 5):

$$k = Aexp(-Ea/RT) \tag{4}$$

or
$$lnk = lnA - (Ea/RT) \tag{5}$$

where, k is the reaction rate constant value, A the Arrhenius constant, Ea (kJ.mol^{-1}) the activation energy, R (8.31 J.mol^{-1}K^{-1}) the universal gas constant and T (K) the absolute temperature.

When lnk is plotted versus the reciprocal of the absolute temperature, a linear relationship should be observed in the temperature range studied. The slope of the line obtained permitted to calculate the Ea and the ordinate intercept corresponds to lnA (Dogan et al., 2002). The values of activation energy (Ea) and Arrhenius constant (A) allowed the determination of different thermodynamic parameters such as variations in enthalpy ($\Delta H^{\#}$), entropy ($\Delta S^{\#}$) and Gibbs free energy ($\Delta G^{\#}$) according to following equations (Eq. 6; 7; 8):

$$\Delta H^{\#} = Ea - RT \tag{6}$$

$$\Delta S^{\#} = R\ (lnA - lnKB\ /\ h - lnT) \tag{7}$$

$$\Delta G^{\#} = \Delta H^{\#} - T\Delta S^{\#} \tag{8}$$

where, KB (1.38 x 10^{-23} J.K^{-1}) is the Boltzmann's constant, h the Planck's constant (6.626 x 10^{-34} J.s) and T the absolute temperature.

2.7 Statistical Analyses

Statistical analyses were carried out in triplicate. Results were expressed as means ± standard deviation. The statistical differences among the means of data were calculated using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Significance was set at $P < 0.05$.

3. Results and Discussion

3.1 Thermal Inactivation Kinetics of Carboxylmethylcellulases

We have earlier reported that a new endo-beta-D-glycosidase from salivary glands of *Macrotermes subhyalinus* little soldier with a dual activity against carboxymethylcellulose and xylan has also been isolated and partially described (Fagbohoun, et al., 2012). In this study, the effect of heat treatment over a range of temperature from 50 to 65°C on both carboxymethylcellulases Ab-CX1 and Ab-CX2 was evaluated by determining the residual percentage activity (Table 1).

Table 1. Effect of treatment temperature and time on inactivation of carboxymethylcellulases Ab-CX1 and Ab-CX2 from little soldier of *Macrotermes subhyalinus*

Time (min)	Residual activity (%) at each temperature (°C) of heat treatment							
	50		55		60		65	
	Ab-CX1	Ab-CX2	Ab-CX1	Ab-CX2	Ab-CX1	Ab-CX2	Ab-CX1	Ab-CX2
5	95.12 ± 5.11	91.39 ± 3.19	90.48 ± 5.90	91.39 ± 2.34	87.80 ± 4.18	83.96 ± 2.89	74.23 ± 1.52	78.66 ± 2.19
10	90.48 ± 2.53	86.07 ± 3.12	81.55 ± 2.11	76.33 ± 0.74	77.88 ± 1.89	71.69 ± 4.17	63.76 ± 1.45	65.96 ± 2.14
15	84.44 ± 2.89	81.05 ± 0.65	74.08 ± 4.51	67.03 ± 2.38	70.46 ± 3.22	63.76 ± 1.24	54.88 ± 2.17	54.39 ± 1.47
20	81.87 ± 4.51	73.34 ± 1.22	70.46 ± 4.41	60.56 ± 4.57	63.76 ± 2.17	49.65 ± 1.98	45.84 ± 1.22	41.51 ± 1.31
25	76.33 ± 2.88	67.70 ± 2.82	63.12 ± 2.87	54.88 ± 2.38	58.86 ± 1.59	42.74 ± 3.15	36.83 ± 0.76	33.92 ± 1.68
30	74.08 ± 0.34	63.73 ± 1.66	59.45 ± 1.59	49.65 ± 2.67	51.68 ± 2.55	36.78 ± 1.52	30.11 ± 1.59	29.81 ± 2.55
35	70.46 ± 1.26	59.89 ± 2.92	54.88 ± 1.14	41.89 ± 1.75	47.23 ± 2.68	30.11 ± 2.36	25.92 ± 2.33	22.31 ± 1.17
40	67.03 ± 2.60	55.49 ± 1.45	49.46 ± 1.67	38.67 ± 2.07	42.74 ± 3.19	24.65 ± 1.37	22.31 ± 2.51	18.26 ± 0.25
45	62.96 ± 1.03	51.17 ± 0.34	44.93 ± 2.55	33.28 ± 4.21	38.67 ± 0.11	20.18 ± 1.96	16.52 ± 1.81	14.95 ± 0.56
50	59.45 ± 0.25	47.71± 2.01	41.89 ± 2.31	30.11 ± 1.49	34.30 ± 1.22	18.26 ± 0.52	13.53 ± 0.17	12.24 ± 1.22
55	56.55 ± 2.17	43.60 ± 1.01	38.67 ± 2.29	27.25 ± 2.92	30.11 ± 1.38	14.95 ± 1.33	11.08 ± 0.25	10.02 ± 1.05
60	53.79 ± 1.76	40.56 ± 3.52	36.05 ± 3.15	24.65 ± 3.17	28.08 ± 0.25	12.24 ± 3.11	10.02 ± 1.01	9.11 ± 0.11

The obtained values are mean ± SD of three independent determinations. On the lines of each parameter, the averages affected of no common letter are significantly different between them on the threshold of 5% according to the test of Duncan.

Thus, we note an enzyme activity peak usually referred to the optimum temperature and which varies for different enzymes (Trasar-Cepeda et al., 2007). The activity of both carboxylmethylcellulases was decreased with increasing heating time (5-60 min) and temperature (50-65°C). Indeed, between 50 to 65°C, heat-denaturation of Ab-CX1 and Ab-CX2 occurred after incubation for 5 min (95.12 ± 5.11 to 74.23 ± 1.52 % and 91.39 ± 3.19 to 78.66 ± 2.19 %, respectively). Thus, the heat treatment at 50°C during 60 min caused a partial inactivation of 53.79 ± 1.76 % for Ab-CX1 and 40.56 ± 3.52 % for Ab-CX2. A partial inactivation of 51.68 ± 2.55 and 36.78 ± 1.52 % was also observed for Ab-CX1 and Ab-CX2, respectively, after heating at 60°C during 30 min. However, a strong inactivation of both enzyme activities was obtained after 60 min of heat treatment at 65°C. Moreover, carboxylmethylcellulases from abdomen of *Macrotermes subhyalinus* little soldier showed a typical temperature-dependent inactivation profile in the presence of the substrate used. At higher temperature, the enzyme most likely underwent denaturation and lost its activity. Stauffer (1989) states that denaturation is the heat induced spontaneous, irreversible breakdown of the secondary and tertiary structure of the enzyme protein such that the enzyme will no longer function and cannot re-activate. The results of the heat inactivation studies suggest that these enzymes belong to the group of thermostable enzymes. Compared to Ab-CX2, results show that Ab-CX1 was the most thermostable because it retained about 50 and 55 % activity after 30 and 15 min at 60 and 65°C, respectively. Based on the semi-log plots linear of carboxylmethylcellulase activities versus heat treatment time at temperature ranged from 50 to 65°C (Table 1), it can be concluded that thermal inactivation described a first-order reaction. These results are in agreement with those reported for peptide cerein 8A (Lappe et al., 2009) and for beta-glucosidase from the digestive juice of the land crab *Cardisoma armatum* (Ya et al., 2014).

The inactivation rate constant (k) value and half-life ($t_{1/2}$) of carboxymethylcellulases Ab-CX1 and Ab-CX2 from little soldier of *Macrotermes subhyalinus* are presented in Table 2.

Table 2. k-values and $t_{1/2}$ for thermal inactivation of carboxymethylcellulases Ab-CX1 and Ab-CX2 from little soldier of *Macrotermes subhyalinus* at temperature range (50-65°C)

Temperature (°C)	Ab-CX1		Ab-CX2	
	k-values (min^{-1})	$t_{1/2}$ (min)	k-values (min^{-1})	$t_{1/2}$ (min)
50	0.0103 ± 0.0003[d]	67.28 ± 2.06[g]	0.0149 ± 0.0007[ab]	46.51 ± 1.98[c]
55	0.0174 ± 0.0004[c]	39.82 ± 1.92[f]	0.0239 ± 0.0005[e]	28.99 ± 0.95[b]
60	0.0215 ± 0.0001[e]	32.23 ± 1.05[e]	0.0340 ± 0.0001[h]	20.02 ± 1.71[a]
65	0.1217 ± 0.0005[b]	17.63 ± 0.07[a]	0.0416 ± 0.0003[de]	16.65 ± 0.04[f]

Results showed that the rate of k-value increased, indicating the thermostabilizing nature of carboxymethylcellulose, as a lower rate constant means the enzyme is more thermostable (Marangoni, 2002). The half-life ($t_{1/2}$) is another parameter that plays an important role in the characterization of enzyme stability (Arogba et al., 1998). As shown in Table 2, $t_{1/2}$ determinations are more accurate and reliable on thermostability. With the increasing temperature, the $t_{1/2}$ decreased and showed values ranged between 67.28 ± 2.06 and 17.63 ± 0.07 min for Ab-CX1, and between 46.51 ± 1.98 and 16.65 ± 0.04 min for Ab-CX2. This would indicate that the enzymes are unstable at higher temperature (Lappe, et al., 2009). The increase in half-life by 1.6 fold for Ab-CX1 at 60°C clearly indicates that Ab-CX1 was more stable. Also, the decimal reduction time (D-value) needed for 90% reduction of the initial enzyme activity was calculated.

The corresponding D-values for Ab-CX1 and Ab-CX2 are given in Table 3. D-values decreased with increasing temperature from 50 to 65°C, indicating a faster inactivation of carboxylmethylcellulases at higher temperatures. Similarly to the work of Sant'anna et al. (2011), thermal stability of peptide P34 decreased at higher temperature. D-values for Ab-CX1 and Ab-CX2 ranged from 223.59 ± 4.74 to 58.60 ± 0.96 min and 154.56 ± 2.64 to 55.36 ± 2.02 min, respectively. It should be noted that at 60°C, the D-value for Ab-CX1 inactivation was 2 times higher than the corresponding value for Ab-CX2 inactivation. This is probably due to the relative higher thermal stability of Ab-CX1.

The effect of temperature on D- and Z-values of carboxymethylcellulases Ab-CX1 and Ab-CX2 from little soldier of *Macrotermes subhyalinus* are shown in Table 3.

Table 3. D-, Z- and Ea-values for thermal inactivation of carboxymethylcellulases Ab-CX1 and Ab-CX2 from little soldier of *Macrotermes subhyalinus* at temperature range (50-65°C)

| Kinetic parameters	Ab-CX1	Ab-CX2
D-values (min)		
D_{50}	223.59 ± 4.74^f	154.56 ± 2.64^d
D_{55}	132.35 ± 1.92^j	96.35 ± 3.75^e
D_{60}	107.11 ± 2.94^k	66.56 ± 1.54^i
D_{65}	58.60 ± 0.96^b	55.36 ± 2.02^d
Z-value (°C)	24.21 ± 1.92^a	33.33 ± 2.78^b
Ea (kJ.mol^{-1})	76.74 ± 1.98^b	62.80 ± 2.05^c

The Z-values were calculated and found to be 24.21 ± 1.92 and 33.33 ± 2.78 °C for Ab-CX1 and Ab-CX2, respectively at 50-65°C. Thus the Z-value of Ab-CX1 was lower compared to that of Ab-CX2. The Z-values for cooking and nutrients degradation (25-45°C) are generally greater than microbial inactivation (7-12°C) (Awuah et al., 2007). In fact, differences between the D- and Z-values of enzyme and nutrients are exploited to optimize thermal processes and can be exploited also to maintain carboxymethylcellulases activity after treatment. This indicates that any change in temperature processing affects more intensely the stability of Ab-CX1 than Ab-CX2. In this study, D-, Z- and k-values indicate that both carboxylmethylcellulases are heat stable and then can be used in high temperature short time (HTST) and low temperature long time (LTLT) industrial processes such as pasteurization, where values of 65°C for 3-5 min and 55°C for 30 min, respectively, are generally considered. In fact the D- and Z-values of carboxylmethylcellulases are exploited to optimize thermal processes and to preserve enzyme activity after treatment. According to Barrett et al. (1999), high Z-values indicate more sensitivity to the heat treatment time and low Z-values indicate more sensitivity to increasing temperature.

In order to determine the thermodynamic parameters for thermal stability, the energy of activation (Ea) for thermal denaturation was determined by applying the Arrhenius plot. The Ea can be seen as the energy absorbed or released needed to the molecules be able to react (Van Boekel, 2008). In this study, from 50 to 65°C, the carboxylmethylcellulase activation energy values were calculated to be 76.74 ± 1.98 and 62.80 ± 2.05 kJ.mol^{-1} for Ab-CX1 and Ab-CX2, respectively (Table 3). Thus, Ab-CX1 in the presence of carboxymethylcellulose substrate displayed relatively higher energy barrier (76.74 ± 1.98 kJ.mol^{-1}) than Ab-CX2 (62.80 ± 2.05 kJ.mol^{-1}). Obviously, Ab-CX2 showed a considerably higher thermosensitivity upon heat treatment. These values were lower than those of endoglucanase from *Humicolain solens* (108.69 kJ.mol^{-1}) and of beta-glucosidase from *Cardisoma armatum* (172.98 kJ.mol^{-1}) (Riaz et al., 2014; Ya, et al., 2014). However, they were higher than an intracellular beta-glucosidase from a mutant-derivative of *C. biazotea* (57 kJ.mol^{-1}) (Rajoka et al., 2004). Both carboxylmethylcellulases (Ab-CX1 and Ab-CX2) had high relative activation energy values, which could indicate an increased stability at higher temperatures and that the enzyme conformation was still stable at these

temperatures (Leite et al., 2007). The higher value of *Ea* means more energy is required to denature the treated enzyme as postulated by Tayefi-Nasrabadi and Asadpour (2008).

3.2 Thermodynamic Studies of Carboxylmethylcellulases

Thermostability represents the capability of an enzyme molecule to resist against thermal unfolding in the absence of substrate, while thermophilicity is the ability of an enzyme to work at elevated temperatures in the presence of substrate (Georis et al., 2000; Sarath Babu et al., 2004; Bhatti et al., 2013). Thermal inactivation may occur in two steps as shown below:

$$N \leftrightarrow U \to I$$
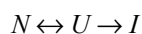
Where N is the native, U is the unfolded enzyme, which could be reversibly refolded upon cooling, and I is the inactivated enzyme formed after prolonged exposure to heat, and therefore, cannot be recovered on cooling. The thermal denaturation of enzymes is accompanied by the disruption of non-covalent linkages, including hydrophobic interactions, with concomitant increase in the enthalpy of activation (Srivastava et al., 2005). The opening up of the enzyme structure is accompanied by an increase in the disorder, randomness or entropy of activation (Vieille & Zeikus, 1996).

In the study of the mechanism of thermal inactivation of proteins, valuable information can be obtained by identifying some inactivation parameters, such as enthalpy ($\Delta H^{\#}$), entropy ($\Delta S^{\#}$) and Gibbs free energy ($\Delta G^{\#}$). Thus, the determination of these thermodynamic parameters was carried out by measuring the carboxylmethylcellulase activities at different temperatures (50-65°C). Table 4 shows these thermodynamics parameters for carboxylmethylcellulases Ab-CX1 and Ab-CX2.

Table 4. Thermodynamic parameters of carboxymethylcellulases Ab-CX1 and Ab-CX2 from little soldier of *Macrotermes subhyalinus* under heat treatment between 50 and 65°C (assuming a 1st-order kinetic model)

| Temperature | Thermodynamic parameters | | | | | |
| | $\Delta H^{\#}$ (kJ.mol^{-1}) | | $\Delta S^{\#}$ (J.mol^{-1}K^{-1}) | | $\Delta G^{\#}$ (kJ.mol^{-1}) | |
(°C)	Ab-CX1	Ab-CX2	Ab-CX1	Ab-CX2	Ab-CX1	Ab-CX2
50	74.06 ± 0.02[f]	60.12 ± 0.02[d]	190.10 ± 0.01[n]	21.72 ± 0.02[a]	12.63 ± 0.02[e]	53.10 ± 0.02[d]
55	74.02 ± 0.01[e]	60.08 ± 0.01[e]	189.97 ± 0.02[m]	21.59 ± 0.02[b]	11.68 ± 0.01[f]	52.99 ± 0.02[e]
60	73.98 ± 0.01[h]	60.04 ± 0.01[c]	189.85 ± 0.02[k]	21.46 ± 0.01[c]	10.73 ± 0.02[g]	52.88 ± 0.01[b]
65	73.93 ± 0.02[g]	59.99 ± 0.02[b]	189.72 ± 0.01[l]	21.34 ± 0.02[d]	9.78 ± 0.01[h]	52.78 ± 0.02[c]
Mean	74.00 ± 0.03[k]	60.06 ± 0.01[l]	189.91 ± 0.03[k]	21.53 ± 0.01[b]	11.20 ± 0.01[f]	52.94 ± 0.02[e]

The average values of $\Delta H^{\#}$, $\Delta S^{\#}$ and $\Delta G^{\#}$ were respectively 74.00 ± 0.03 kJ.mol^{-1}, 189.91 ± 0.03 J.mol^{-1}K^{-1} and 11.20 ± 0.01 kJ.mol^{-1} for Ab-CX1 and 60.06 ± 0.02 kJ.mol^{-1}, 21.53 ± 0.01 J.mol^{-1}K^{-1} and 52.94 ± 0.02 kJ.mol^{-1} for Ab-CX2. Results also show that the Ab-CX1 enthalpy was higher than that of Ab-CX2. The high enthalpy ($\Delta H^{\#}$) change in the system clearly indicates that more energy is required for thermal denaturation of enzyme (Bhatti et al., 2005). The observed change in $\Delta H^{\#}$ also indicates that enzyme undergoes considerable change in conformation at higher temperatures even after treatment (Marín et al., 2003). In this study, the positive value of this parameter indicates that the catalytic reaction is endothermic.

According to Anema and Mckenna (1996), the positive values of entropy ($\Delta S^{\#}$) for the hydrolysis reaction of carboxymethylcellulose indicate that the reaction proceeds with less speed and is characterized by low regularity. Small changes in the values of $\Delta S^{\#}$ indicates a preferential destruction of weak bonds (hydrogen and electrostatic), resulting in a lower loss of catalytic activity. The positive values for change in $\Delta S^{\#}$ also indicate that there are no significant processes of aggregation for both carboxylmethylcellulases. Furthermore, the high values obtained for $\Delta S^{\#}$ variation probably reflect an increased disorder of the active site or the structure of each carboxylmethylcellulase, which is the main driving force of heat denaturation (D'amico et al., 2003). Generally, activation entropy has a dominant role in thermal inactivation of proteins in aqueous solutions (Bromberg et al., 2008).

The Gibbs free energy change ($\Delta G^{\#}$) indicates the spontaneity of the reaction catalyzed under the conditions of temperature and pressure used. In this study, $\Delta G^{\#}$ values were positive, indicating that the processes were endergonic and not spontaneous.

4. Conclusion

Based on an isothermal experiment in the temperature range from 50 to 65°C and using Arrhenius equation, the thermal inactivation of carboxymethylcellulases Ab-CX1 and Ab-CX2 can be explained by the first-order model. The D-, Z-, k-values, indicate that Ab-CX1 and Ab-CX2 are heat stable and then could be utilized in pasteurization conditions, maintaining part of their biological activity. The high values obtained for activation energy (Ea) and change in enthalpy ($\Delta H^{\#}$) indicated that a high amount of energy was needed to initiate denaturation of these carboxylmethylcellulases, most likely due to its stable molecular conformation.

Acknowledgments

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